OUTLINE OF THE PROPOSED WORK FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

A SYNOPSIS

"Isolation, Docking and Screening of Phytoconstituent from some Antidiabetic plants with Bovine serum albumin"

Submitted for the consideration of

Registration for the Degree of *Doctor of Philosophy*

In the Faculty of Pharmaceutical Sciences and Technology,

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1. Abstract:

Diabetes Mellitus is a multifunctional disease leading to the several complications and thus, demanding for a therapeutic approach. Medicinal plants with several antidiabetic compounds play an important role in treating diabetes. The serum albumin is most abundant protein in plasma and has high affinity to bind drug ligands and metabolites, thus, acting as a carrier for them. This capability of serum albumin makes it vital to play a function in certain physiological processes such as distribution and transport of various ligands. The ligands bind to albumin either weakly or strongly and the type of binding will have impact on the distribution of these ligands as weakly bound ligands will have poor distribution and fast elimination and the strongly bound ligands will decrease the free ligand amount in plasma. To understand the PK and PD of drug molecules there is need to investigate the behavior of binding between the drug molecules and albumin. Considering the side effects and exorbitant cost of the many current medicines, in the past few years, herbal medicines are gaining momentum in treating various diseases because of their natural origin and lee or no side effects.

In this view, it is felt worth to isolate, dock and screen phytoconstituents from few plants for possible antidiabetic activity.

2. Introduction:

Diabetes Mellitus is a metabolic disorder of proteins, fats and carbohydrate metabolism which is characterized by high fasting and post prandial blood sugar levels. Diabetes mellitus results either from less production of insulin or insulin dysfunction [1]. The disease is estimated to increase from 4% in the year 1995 to 5.4% by the year 2025 [2]. Early diagnosis and adequate treatment is very important for management of the disease. Complications include diabetic ketoacidosis, coronary heart diseases, macro-angiopathy, micro-angiopathy, neuropathy, retinopathy, cataracts and renal failure [3, 4].

The serum albumin is most abundant protein in plasma and has high affinity to bind drug ligands and metabolites, thus, acting as a carrier for them. This capability of serum albumin makes it vital to play a function in certain physiological processes such as distribution and transport of various ligands. The ligands bind to albumin either weakly or strongly and the type of binding will have impact on the distribution of these ligands as weakly bound ligands will have poor distribution and fast elimination and the strongly bound ligands will decrease the free ligand amount in plasma. To understand the PK/PD of drug molecules there is a need to investigate the behavior of binding between the drug molecules and albumin. Bovine serum albumin (BSA) is structurally analogous to the human serum albumin (HSA), and both of them have been widely studied for their interaction with drug ligands. The studies include multi-spectroscopic and molecular simulation approach with theoretical calculations [5].

Nearly 21,000 plants have been listed by the World Health Organization, which have numerous medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale [6].

Considering the side effects and exorbitant cost of the many current medicines, in the past few years, herbal medicines are gaining momentum in treating various diseases because of their natural origin and less or no side effects.

3. Literature Review:

Extensive literature survey was carried out to found out the methods of isolation of of phytoconstituent, molecular modeling, computer simulation including docking studies of the natural compounds with Bovine Serum Albumin and various screening methods for it.

1. **Fazeela Mehaboob Begum SM et al (2017)**

   The qualified herbal ligand Andrographolide has high binding potential against the target protein receptor, PPAR γ . The resulting ligand was also found to be fit with good druggable character, as per Lipinski's rule of five. Thus, this ligand could be a potential drug candidate for treating diabetes mellitus. The resulting medicinal plant was subjected to in vitro analysis where phytochemical evaluation, anti-oxidant activity, anti-diabetic activity, anti-inflammatory activity and the anti-glycation activity of the five different solvent extracts were determined. The results obtained indicated that the extracts possess a significant level of activity and are concentration dependent.

2. **Tanveer A. Wani et al (2017)**

   Rivaroxaban quenched the basic fluorescence of BSA molecule by the process of static quenching since rivaroxaban and BSA form a complex that results in shift of the absorption spectra of BSA molecule. A decline in the values of binding constants was detected with the increase of temperatures (298–308 K) and the binding constants were in range from 1.32 × 105 to 4.3 × 103 L mol−1 indicating the instability of the BSA and rivaroxaban complex at higher temperatures. The data of number of binding sites showed uniformity. The site marker experiments indicated site I (sub-domain IIA) as the principal site for rivaroxaban binding. The thermodynamic study experiments were carried at the temperatures of 298/303/308 K. The ΔG0, ΔH0 and ΔS0 at these temperatures ranged between − 24.67 and − 21.27 kJ mol−1 and the values for ΔH0 and ΔS0 were found to be − 126 kJ mol−1 and ΔS − 340 J mol−1 K−1 The negative value of ΔG0 indicating spontaneous binding between the two molecules. The negative values in ΔH0 and ΔS0 indicated van der Waals interaction and hydrogen bonding were involved during the interaction between rivaroxaban and BSA.

3. **Mihaela Skrt et al (2012)**

   Authors have investigated the binding of several polyphenols to bovine serum albumin (BSA) at pH 7.5 and 25 C: catechins [()-epigallocatechin-3-gallate, ()-epigallocatechin, ()-epicatechin-3-gallate],

flavones (kaempferol, kaempferol-3-glucoside, quercetin, naringenin) and hydroxycinnamic acids (rosmarinic acid, caffeic acid, p-coumaric acid). Fluorescence emission spectrometry and molecular docking were applied to compare experimentally determined binding parameters with molecular modelling. Among these polyphenols, ()-epicatechin-3-gallate showed the highest Stern–Volmer modified quenching constant, followed by ()-epigallocatechin-3-gallate. Similarly, ()-epicatechin-3-gallate had the highest effect on the Circular Dichroic spectrum of BSA, while the changes induced by other polyphenols were negligible. Molecular docking predicted high binding energies for epicatechin-3-gallate and epigallocatechin-3-gallate for the binding site on BSA near Trp213.

4. **Mingxiong Tan et al (2010)**

   Authors have reported the interaction of evodiamine (Evo) with bovine serum albumins (BSAs) at different two temperatures (298 and 310 K) by means of fuorescence spectroscopy. The experimental results showed that Evo binds with BSA via a static quenching procedure with association constants of 1.61 × 106 L/mol at 298 K and 6.78 × 105 L/mol at 310 K. The number of bound Evo molecules per protein is 1.31 at 298 K and 1.33 at 310 K. Results suggested that Evo reacts with BSA chiefly through hydrophobic and electrostatic interactions, and it does not alter the α-helical nature of BAS.

5. **Md Rokonujjaman et. al., (2015)**

   In present investigation the quenching of BSA by SC was increased in presence of BF and MH but remained close in presence of both BF and MH. Quenching constants were larger for the BSA-(SC+BF) system and ranked in the order as BSA-(SC+BF)>BSA-(SC+MH)>BSA-(SC+BF+MH)≈ BSA-SC at 280 nm at two different temperatures, respectively. But quenching at the excitation wavelength of 293 nm was ranked in order as BSA-(SC+BF) >BSA-(SC+MH) >BSA-(SC+BF+MH)>BSA-SC at 298 K and 308 K, respectively.

4. Aim and Objective:

Aim:

Diabetes Mellitus is a multifactorial disease leading to the several complications and thus, demanding for a therapeutic approach. Medicinal plants with several anti-diabetic compounds play an important role in treating diabetes. The serum albumin is most abundant protein in plasma and has high affinity to bind drug ligands and metabolites, thus, acting as a carrier for them. This capability of serum albumin makes it vital to play a function in certain physiological processes such as distribution and transport of various ligands.

In view of the fact it was felt worth to isolate, dock and screen few phytoconstituents from some antidiabetic plants with Bovine Serum Albumin.

Objectives:

The present work would be undertaken with the following objectives.

1. To collect some medicinal plants with proven antidiabetic potential.
2. To extract phytoconstituents from the plant using various methods of extraction.
3. To isolate and characterize the phytoconstituent.
4. To study the interaction of isolates with Bovine Serum Albumin using virtual docking method.
5. To characterize the interaction using fluorescence quenching and UV spectroscopy.
6. To screen the isolates with BSA for possible antidiabetic activity *in vitro*.

5. Methodology to be adopted:

a. Collection of plant material:

Two plants are selected namely *Bougainvillea spectabilis* and *Feronia limonia*. The plants selected for the present study would be collected from Latur District.

b. Extraction of Phytoconstituent:

The selected plants would be processed for successive solvent extraction technique.

c. Isolation and Characterization of isolate:

The extract will be subjected for isolation of the phytoconstituent using TLC, preparative TLC and column chromatography. The isolates will be characterized using various analytical methods like UV, FTIR, 1HNMR, 13CNMR and Mass Spectroscopy.

d. Docking Study:

The isolates would be subjected to virtual docking study for revealing the interactions of isolates with the Bovine Serum Albumin (PDB *id*- 4OR0). The docking study would be carried out using Schrodinger Molecular Interface, LLC. Maestro Modul will be used to study possible interactions.

e. Fluorescence Quenching and UV spectroscopy:

To explore the changes in the structure and conformation of isolates and BSA complex UV absorption spectroscopy will utilized. Fluorescence quenching studies to explore the binding interaction of drug ligands with proteins is considered as the best methodology.

f. Screening of complex for antidiabetic activity:

Inhibition of alpha amylase assay: Alpha-amylase is an enzyme helpful in breaking down large insoluble starch molecules into absorbable molecules and is found in saliva and pancreatic juice.

6. Importance of study /society application:

BSA has been extensively used for such investigations because of its intrinsic structural similarity to human serum albumin. BSA has a wide range of physiological functions that are associated with binding, transport and distribution of biologically active compounds. The binding of active compounds to serum albumins influences the effectiveness of the biologically active compounds, as well as the activity of the serum albumins.

As Diabetes Mellitus is epidemic throughout the world, effective drugs with less/no toxicity need to be developed. And one such way is the use of herbal medicines which will have no side effects.

7. Proposed work plan:

Sr. no	Activity	Months
1.	Literature survey	3
2.	Selection and Collection of plant	3
3.	Extraction of phytoconstituent	6
4.	Isolation and characterization of isolates	12
5.	Virtual Docking study of isolate	3
6.	Fluorescence quenching and UV analysis	3
7.	Screening of isolates for antidiabetic activity	3
8.	Submission of report	3
	Total	36

8. References:

1. Lei L, Ying M, Run-Ling W, Wang SQ, et al. (2013) Find novel dual agonist drugs for treating type 2 diabetes by means of chemi-informatics. Drug Des Devel Ther 7: 279–288.
2. Ramachandran A, Snehalatha C, Viswanathan V (2002) Burden of type 2 diabetes and its complications- the Indian scenario. Curr Sci 83: 1471–1476.
3. Manisha M, Priyanjali D, Jayant L, Ghaskadbi S, Thomas PAD (2007) Indian herbs and herbal drugs used for the treatment of Diabetes. J Clin Biochem Nutr 40: 163–173.
4. Sonawane L.V., Bari S. B. PPAR-γ Novel Target in Antidiabetic Drug Design, International J of Drug Design and Discovery2010, 1(2), 155-160.
5. Tanveer A. Wani, Haitham AlRabiah, Ahmed H. Bakheit, Mohd Abul Kalam and Seema Zargar Study of binding interaction of rivaroxaban with bovine serum albumin using multi-spectroscopic and molecular docking approach, Chemistry Central Journal201711:134.
6. Seth SD, Sharma B (2004) Medicinal plants of India. Ind J Med Res 120: 9‑11.
7. Fazeela Mehaboob Begum SM1, Zakia Fathima S1, Priya S1, Sundararajan R2 and Hemalatha S, Screening Indian Medicinal Plants to Control Diabetes ‑ An *In silico* and *In vitro* Approach, Gen Med (Los Angeles) 2017, 5:2, DOI: 10.4172/2327-5146.1000289.
8. Mihaela Skrt, Evgen Benedik, Crtomir Podlipnik, Nataša Poklar Ulrih, Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking, Food Chemistry 135 (2012) 2418–2424. DOI:10.9734/BJMMR/2015/11655
9. Mingxiong Tan, Weijiang Liang,1 Xujian Luo, and Yunqiong Gu, Fluorescence Spectroscopy Study on the Interaction between Evodiamine and Bovine Serum Albumin, Journal of Chemistry, http://dx.doi.org/10.1155/2013/308054.
10. Md. Rokonujjaman, Md. Abdus Salam and Md. Zakir Sultan, *In vitro* Study of Interactions of Sildenafil Citrate with Bovine Serum Albumin in Presence of Bisoprolol Fumarate and Metformin Hydrochloride by Fluorescence Spectrophotometry, British Journal of Medicine & Medical Research, DOI:10.9734/BJMMR/2015/11655

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